Am J Cancer Res 2023;13(3):758-777
www.ajcr.us /ISSN:2156-6976/ajcr0144733

Original Article

Characterizing the oncogenic importance and exploring gene-immune cells correlation of ACTB in human cancers

Guangyao Li[1], Sehar Samuel[2], Sher Ehsan Ul Haq[3], Ayman S Mubarak[4], Christian R Studenik[5], Asif Islam[6], Mohammed Aufy[5], Mustafa Jawad Kadham[7], Abdul Kareem J Al-Azzawi[8], Mostafa A Abdel-Maksoud[4]

[1]Department of Gastrointestinal Surgery, The Second People's Hospital of Wuhu, Wuhu, Anhui, The People's Republic of China; [2]Tehsil Head Quarter Kotmomin, Kotmomin, Pakistan; [3]Department of Pathology, Faisalabad Medical University, Pakistan; [4]Botany and Microbiology Department, College of Science, King Saud University, Riyadh 11451, Saudi Arabia; [5]Department of Pharmaceutical Sciences, Division of Pharmacology and Toxicology, University of Vienna, Vienna, Austria; [6]Department of Medicine, Mayo Hospital, Lahore, Pakistan; [7]College of Medical Techniques, Al-Farahidi University, Baghdad, Iraq; [8]Al-Turath University College, Baghdad, Iraq

Received June 13, 2022; Accepted January 27, 2023; Epub March 15, 2023; Published March 30, 2023

Abstract: After cardiovascular diseases, cancer is the second deadliest malignancy in the world. The current study was launched to investigate the diagnostic and prognostic landscape of Beta-actin (ACTB) via a multi-layered bioinformatics approach. ACTB expression was analyzed and validated via UALCAN, TIMER, GENT2, GEPIA, and HPA. ACTB promoter methylation was evaluated via MREXPRES. Furthermore, ACTB prognostic values and their correlation with cancer metastasis were explored through the KM plotter and TNMplot, respectively. Then, cBioPortal, CancerSEA, Enrichr, TIMER, MuTarget, and CDT were used to analyze ACTB-related genetic alterations, transcription factors (TFS), MicroRNAs (miRNAs), chemotherapeutic drugs, and the correlation between its expression, immune cells, and different other parameters. We found that ACTB expression was remarkably higher in 24 major human cancer tissues than the normal samples. Additionally, elevated ACTB expression was associated with poorer survival and metastasis in only liver hepatocellular carcinoma (LIHC), head and neck squamous cancer (HNSC), and lung adenocarcinoma (LUAD). This implies that ACTB plays a significant role in the development and progression of LIHC, HNSC, and LUAD. Furthermore, enrichment analysis showed that ACTB-associated genes regulate different Biological Processes (BP), Molecular Functions (MF), and Kyoto Encyclopedia of Genes and Genomes (KEGG) terms. Moreover, ACTB up-regulation had interesting correlations with immune infiltration of CD4+ T, and CD8+ T, tumor purity, mutant genes, and a few other important parameters. At last, via this study, we also explored ACTB-associated clinically important expression regulators, including TFS, miRNAs, and different chemotherapeutic drugs. The results of the present study suggested that ACTB might be a potential candidate biomarker in LIHC, HNSC, and LUAD.

Keywords: ACTB, biomarker, cancer, diagnostic, prognostic, analysis

Introduction

Cancer involves the uncontrolled proliferation and procurement of metastatic properties by the abnormal cells. Cancer is the world's second leading cause of death after cardiovascular diseases [1]. In the United States of America (USA), around two million cancer cases and approximately 610,000 deaths were occurred due to cancer in 2020 [2]. While in 2018, more than 17 million cancer cases and around 10 million deaths were recorded worldwide due to this disease [3]. Furthermore, it is expected that in 2021, the annual number of cancer cases in the USA will rise by 24% in men and by about 21% in women [4]. Cancer is also known as a multi-factorial disease, and different factors such as tobacco consumption, pollutions, an unhealthy diet, exposure to radiation, and aging have been considered to be responsible for the development of this disease [5].

During cancer development, the dysregulation of oncogenes, DNA repair, and tumor suppressor genes leads to abnormal cell cycle progression and inactivation of apoptosis. In compari-

son to benign tumors, malignant tumor metastasizes in different body parts occur due to the down-regulation of cell adhesion receptors [6, 7]. Additionally, metalloprotease membrane activity also helps the metastatic cells to spread further across different body parts. There are several mechanisms involved in the dysregulation of oncogenes, DNA repair, and tumor suppressor genes, and the most crucial mechanisms include genetic mutations, epigenetic changes, copy number variations (CNVs) chromosomal translocation, and deletion [7]. Until now, not enough has been achieved regarding the reduction in new annual cancer cases [8], that's why, the decline in cancer death rate from 2006-2015 has been achieved by only 1.5% annually [8]. Moreover, this achievement in declining cancer cases has come at a huge financial cost. In the future, there are chances that this cost might further increase as the population is increasing rapidly worldwide [9, 10]. Hence, there is an urgent need to identify some novel biomarkers that could help in the detection and treatment of different cancer subtypes as a shared target to reduce cancer-related cases and mortality.

Beta-actin (ACTB) is a housekeeping gene and has widely been used as a control in measuring the expression in various diseases [11]. However, ACTB expression was also found closely related to different cancers, including liver, pancreatic, renal, colorectal, melanoma, prostate, esophageal, lung, gastric, breast, and ovarian cancers. Moreover, the dysregulation and polymerization of ACTB are revealed to be related to the metastasis of different cancers [11]. The current study analyzed the diagnostic value of ACTB and predicted its independent prognostic factor in distinct cancer subtypes using detailed in silico analysis.

Material and methods

The UALCAN database

The UALCAN, which is a dedicated platform for the discovery of novel cancer-associated biomarkers through multi-omics expression analysis of the TCGA cancer datasets [12], was utilized in our study for the pan-cancer gene expression of ACTB to document the differential expression level across multiple cancer types. During analysis, the transcription was normalized as transcript per million (TPM) and for statistics, a t-test ofunequal variance was applied.

KM plotter

We used the Kaplan-Meier plotter tool (http://kmplot.com/analysis/, K-M) for analyzing the prognostic relevancy of ACTB with different cancer subtypes [13]. On the basis of the median value, cancer patients were categorized into two different groups with respect to low and high ACTB. Then, the overall survival (OS) and relapse-free survival (RFS) durations were accessed using the default settings.

TNMplot database

TNMplot (https://www.tnmplot.com/) [14] was conducted in this study to analyze ACTB expression across metastatic tissues relative to control samples. For statistics, a t-test of unequal variance was applied.

Expression validation analysis

TIMER, GENT2, GEPIA, Gene Expression Omnibus (GEO), and Human Protein Atlas (HPA) are recently developed databases for analyzing gene expression data across cancer samples paired with controls [15-19]. Using these databases, we validated the ACTB expression at both transcriptional and translational levels among LIHC, HNSC, and LUAD samples.

MEXPRESS

MEXPRESS [20] was used in this work for computing Pearson correlation between methylation and mRNA levels of the ACTB.

The cBioportal database

The cBioPortal portal, containing numerous datasets from 245 cancer-related studies [21] was used in our research to analyze the ACTB copy number variations (CNVs) and genetic alterations across distinct cancer subtypes.

Costruction of PPI network and enrichment analysis

STRING database is an online software that is dedicated to identifying the known protein

binding partners of the protein(s) of interest [22]. In the present study, we utilized this tool to construct the PPI network of the ACTB-enriched genes. The constructed PPI network was then visualized using Cytoscape software [23]. Furthermore, the enrichment analysis ofthe ACTB-enriched genes was performed through DAVID [24].

Single-cell functional analysis

CancerSEA database has been built to illustrate 14 different functional states of more than 41 thousand cancer cells from 25 tumor types at the single-cell level [25]. We used this database in our study to further assess the oncogenic role of ACTB in LIHC, HNSC, and LUAD at the single cell level.

GEPIA

Via the GEPIA platform, we explored correlations among the ACTB gene expression and its other enriched genes.

MuTarget

MuTarget is designed to explore the associations between expression alterations and genetic mutations across cancer patients [26]. Herein, we conducted MuTarget to identify the possible mutant genes associated with expression alteration in the ACTB gene with default thresh-holds, i.e. $P < 0.05$ and $FC > 1.4$.

Tumor purity, infiltrating level of CD4+ T, and CD8+ T immune cells and ACTB expression in different cancer patients

TIMER database is dedicated to comprehensively analyzing the clinical effects of human different immune cells across distinct cancer subtypes [27]. TIMER used a deconvolution algorithm for predicting the proportion of tumor-infiltrating immune cells (TIICs) of interest with respect to gene expression. We used this database toevaluate the Pearson correlation between ACTB expression and tumor purity, CD4+ T and CD8+ T cells in different cancer patients.

Enrichr database analysis

Enrichr [28] was utilized in this work for exploring ACTB associated miRNAs and TFs.

ACTB gene-drug interaction network analysis

The CTD database [29] was explored in this work to identify ACTB associated drugs.

Results

Transcriptional level of ACTB

Using the UALCAN database, we explored ACTB gene expression in 24 types of cancer tissue paired with controls. As shown in **Figure 1**, all 24 analyzed cancer datasets have highlighted the that mRNA of ACTB was remarkably high in the analyzed cancer subtypes relative to normal individuals, including liver hepatocellular carcinoma (LIHC), head and neck squamous cell carcinoma (HNSC), and lung adenocarcinoma (LUAD) (**Figure 1**).

Predicting prognostic values and metastasis relevancy of ACTB expression

In this study, the KM plotter was interrogated to explore whether ACTB expression was associated with OS and RFS across cancer patients of different types. The results of Log rank test via KM plotter highlighted that ACTB higher expression was associated with poor OS and RFS of the LIHC (HR = 1.58, 95% CI: 1.12-2.24, P = 0.0088, HR = 1.35, 95% CI: 0.91-1.99, P = 0.013), HNSC (HR = 1.69, 95% CI: 1.22-2.35, P = 0.0015, HR = 1.57, 95% CI: 0.72-3.4, P =0.025), and LUAD (HR = 1.58, 95% CI: 1.17-2.13, P = 0.0023, HR = 1.53, 95% CI: 1.01-2.3, P = 0.043) patients (**Figure 2A**, **2B**). Moreover, higher ACTB expression was also noticed to be linked with metastasis in these cancer patients (**Figure 2C**). Therefore, in a nutshell, it is speculated that higher ACTB expression may be a strong prognostic factor and have a significant contribution to the development and progression of LIHC, HNSC, and LUAD.

Relationship between ACTB expression and clinical variables

To explore the association between clinical features and ACTB expression, we utilized the UALCAN platform for investigating different clinical factors of LIHC, HNSC, and LUAD. In **Figure 3**, the box plot highlighted the expression of ACTB based on cancer stage, patients' race,

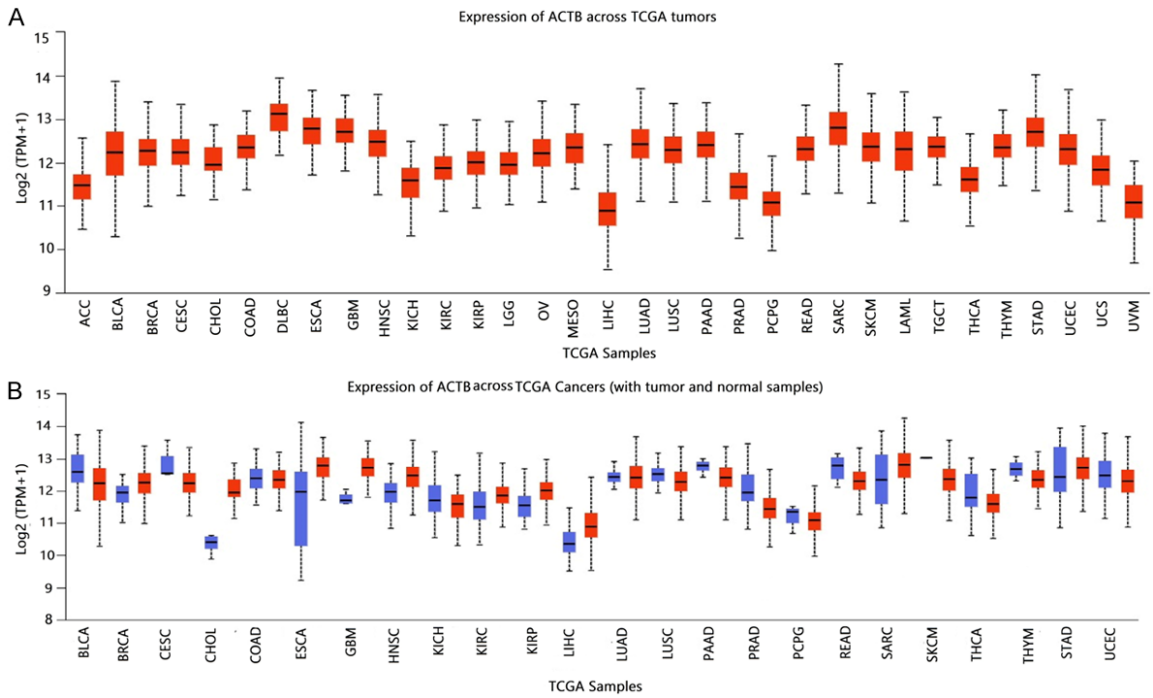


Figure 1. ACTB expression in diverse types of cancer tissues. (A) Only in cancer tissues, (B) In cancer samples and controls. $P < 0.05$.

patients' gender, and nodal metastasis status. The results demonstrated that ACTB has significantly higher expression in LIHC, HNSC, and LUAD patients of different clinicopathological parameters as well relative to controls (**Figure 3**).

Transcription and translation levels validation of ACTB

TIMER, GENT2, GEPIA, GEO and HPA are recently developed databases for analyzing gene expression data across cancer samples paired with controls. With the help of TIMER, GENT2, GEPIA, and HPA databases, we validated the ACTB expression at both transcriptional and translational level among LIHC, HNSC, and LUAD samples as compared to controls on TCGA expression datasets. While, using GEO database, ACTB expression was validated across LIHC, HNSC, and LUAD samples relative to controls on three different datasets i.e., LIHC (GSE45267), HNSC (GSE45216), and LUAD (GSE40791). As shown in **Figure 4**, the expression analysis results from TIMER, GENT2, GEPIA, GEO, and HPA databases reveled that mRNA and protein of ACTB was significantly overexpressed in LIHC, HNSC, and LUAD patients relative to controls at both transcription and translational levels.

Promoter methylation analysis

Using the MEXPRESS database, herein we investigated the correlations among ACTB expression and the promoter methylation of this gene simultaneously across LIHC, HNSC, and LUAD samples. Results of this analysis showed that the ACTB promoter was remarkably hypomethylated across LIHC, HNSC, and LUAD samples as compared to the normal controlls (**Figure 5**).

Genetic alterations analysis of ACTB

Mutations in ACTB across LIHC, HNSC, and LUAD was were checked from LIHC, HNSC, and LUAD TCGA datasets (Firehose Legacy) through cBioPortal database. Results showed that ACTB possess genetic alterations in 0.8% cases of the LIHC, 3% cases of the HNSC with maximum deep amplification, and 8% cases of the LUAD with maximum deep amplification (**Figure 6**).

ACTB PPI network retrieval and enrichment analysis

The ACTB PPI network was retrieved via the STRING database and visualized through

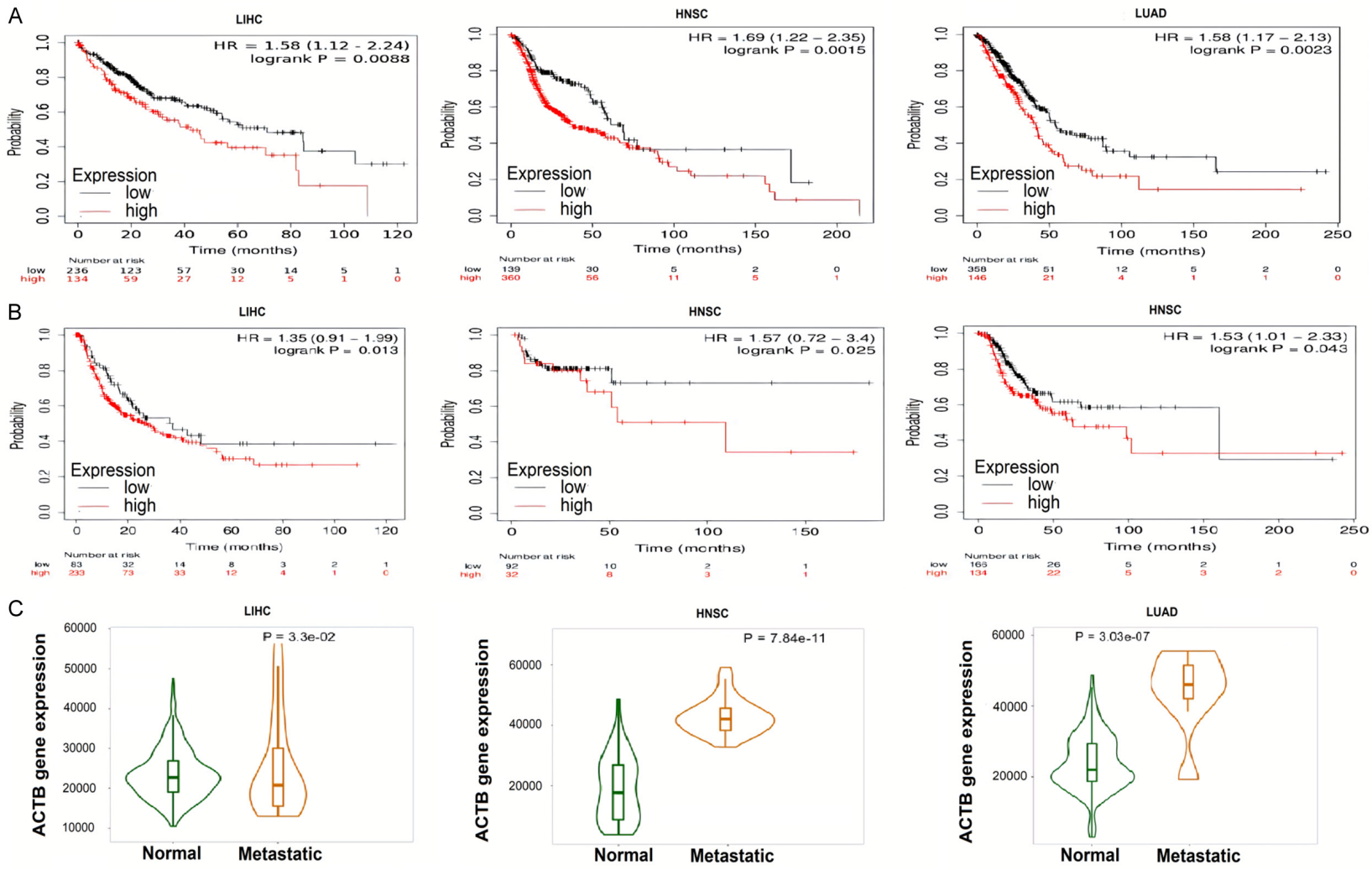


Figure 2. KM curves of ACTB in cancer patients of diverse subtypes. (A) OS curves in cancer patients of LIHC, HNSC, and LUAD, (B) RFS curves in cancer patients of LIHC, HNSC, and LUAD, and (C) A correlation analysis of ACTB with metastasis. $P < 0.05$.

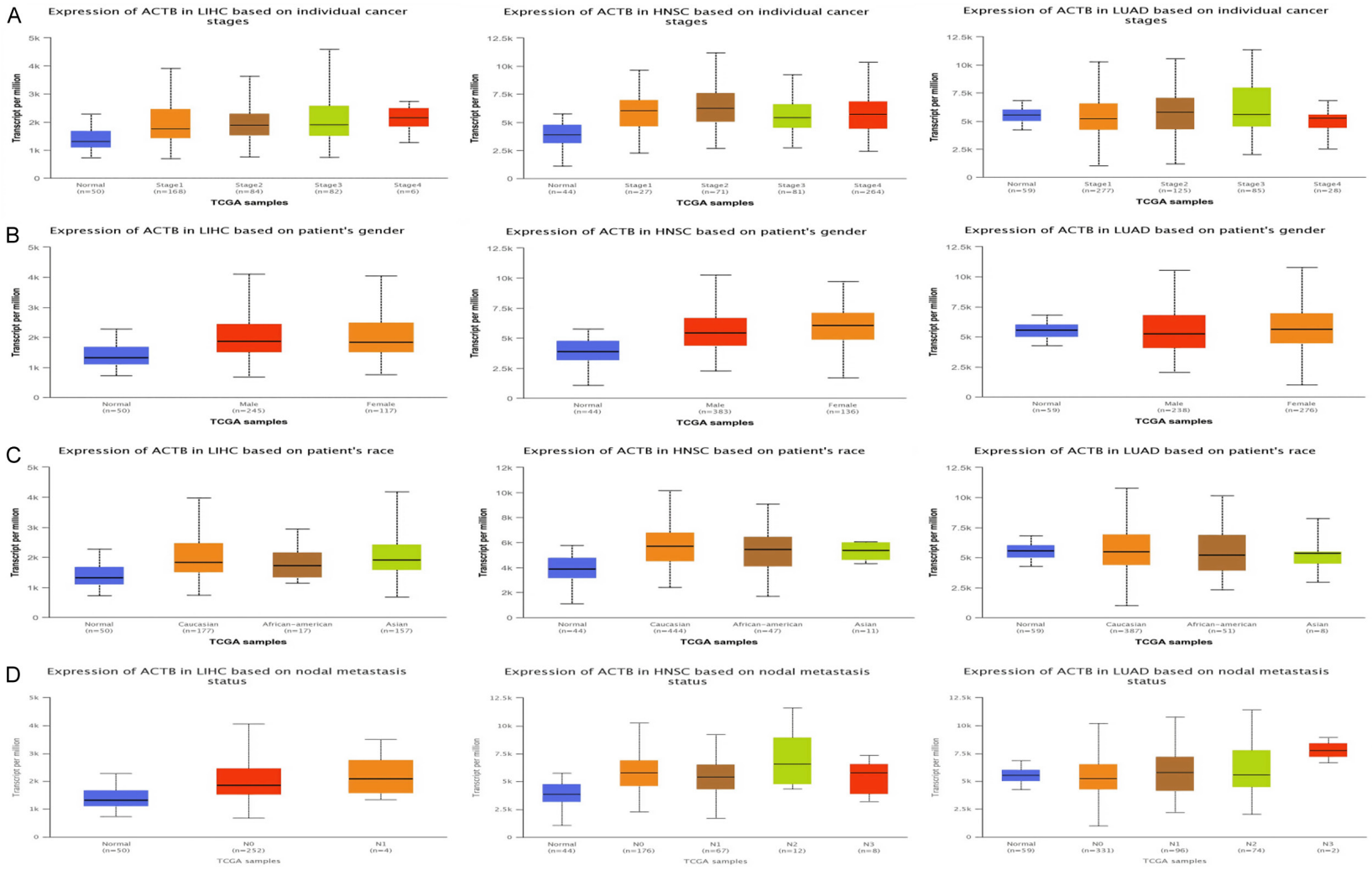


Figure 3. ACTB expression in LIHC, HNSC, and LUAD patients stratified on the basis of cancer stage, race, gender, and nodal metastasis. (A) Cancer stage-based ACTB transcription, (B) Race-based ACTB transcription, (C) Gender-based ACTB transcription in LIHC, HNSC, and LUAD, and (D) Nodal metastasis status-based ACTB transcription. $P < 0.05$.

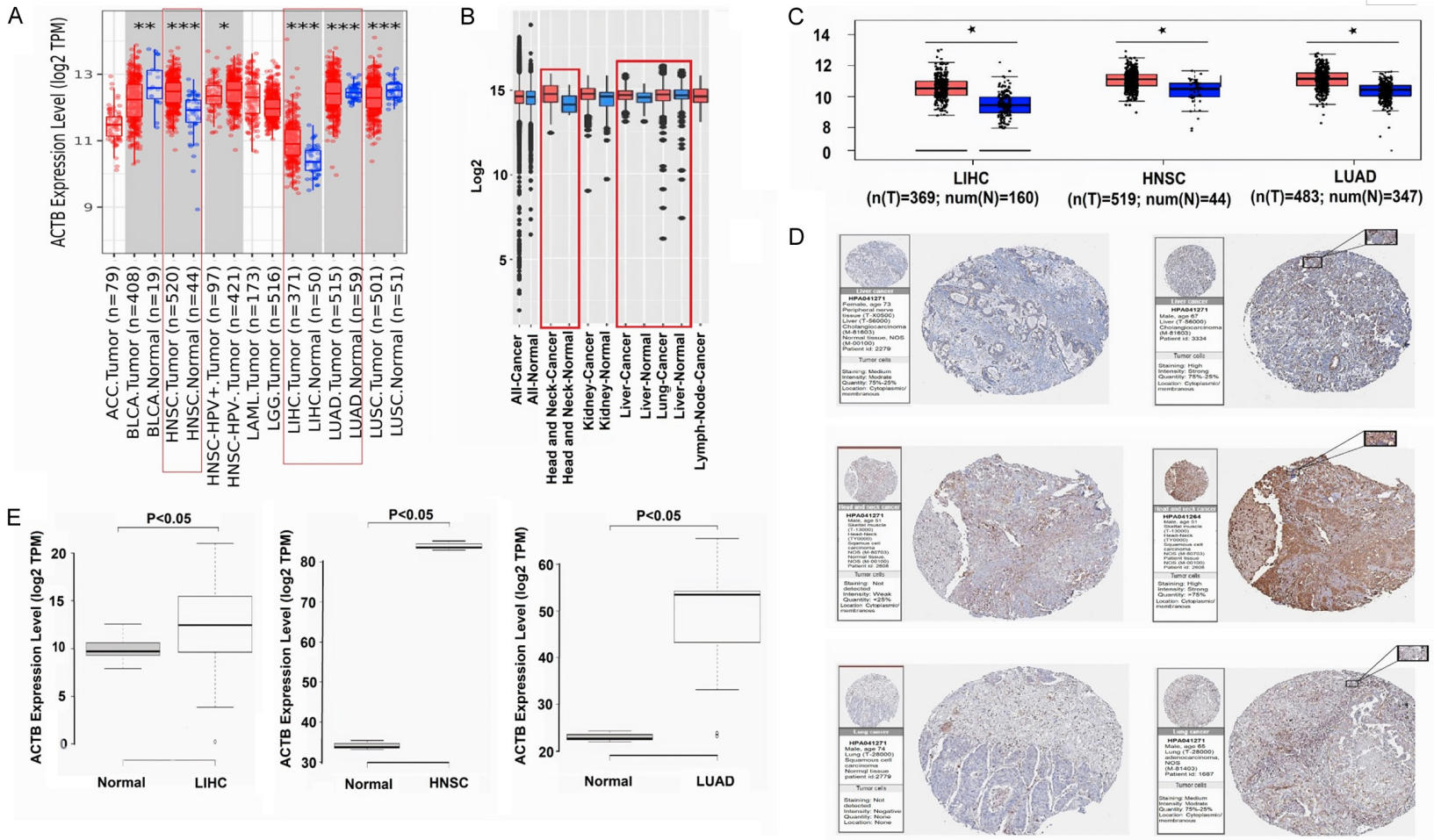


Figure 4. Transcriptional and translational validation ACTB. (A) Via TIMER, (B) Via GENT2, (C) Via GEPIA, (D) Via HPA and (E) Via GEO dataset. $P < 0.05$.

Cytoscape software. ACTB PPI revealed that this gene is mainly enriched in ten other genes (**Figure 7A**). We next subjected the ACTB-enriched genes to GO and KEGG analysis. The results of enrichment analysis have shown the involvement of ACTB-enriched genes in Actin cytoskeleton organization, Box C/D snoRNP assembly, Actin filament depolymerization, Cell junction assembly, and DNA repair biological process (BP) GO terms, Actin monomer binding, ATPase activity, Leucine zipper domain binding, DNA helicase activity molecular (MF) GO terms, and Regulation of actin cytoskeleton, Shigellosis, Salmonella infection, and Rap1 signaling pathway KEGG terms (**Figure 7**; **Table 1**).

Single-cell functional analysis

CancerSEA is a single-cell database, that is used to evaluate different functional states across multiple cancers at the single-cell level, was utilized in this study to further clarify the role of ACTB at the single cell level in LIHC, HNSC, and LUAD cells. In view of our results, ACTB was found to be mainly involved in differentiation and hypoxia in LIHC, metastasis, invasion, hypoxia, EMT in HNSC, and metastasis and EMT in LUAD (**Figure 8**).

Correlation analysis between ACTB and its associated genes expression

Via the GEPIA, we analyzed correlations among ACTB and its other associated gene expressions across LIHC, HNSC and LUAD. The results shown that ACTB expression was positively correlated with expression levels of CDC5L, RUVBL1, ACTG1, ACTC1, CFL2, MKL1, PFN1, CFL1, PFN2, and RUVBL2 in the analyzed cancer types (**Figure 9**).

The correlation between ACTB expression and crucial mutant genes

To identify the mutant genes that could help in LIHC, HNSC, and LUAD therapy and enhance the real hub gene expression, the Mann-Whitney U analysis was carried out to identify mutant genes correlated with ACTB expression. We selected the top 3 mutant genes for ACTB in LIHC, HNSC, and LUAD, respectively, via the MuTarget database. As shown in **Figure 10**, the top 3 mutant genes that positively correlate with the expression of ACTB were DHX8, IQGAP3, and SLC6A11 in LIHC, PCDHB16, TRAF3, and CCDC13 in HNSC, and ZNF572, BPIFB2, and KRT5 in LUAD.

ACTB expression influence the infiltration level of immune cells

We applied TIMER to note whether ACTB has any association with tumor purity and immune cells (CD4+ T and CD8+ T) across LIHC, HNSC, and LUAD. The obtained immune correlations results clearly showed that ACTB expression has an obvious negative correlation with CD8+ T while an obvious positive correlation with CD4+ T immune across LIHC, HNSC, and LUAD samples (**Figure 11A**, **11B**). Furthermore, through TIMER analysis, an obvious negative correlation was also documented in our study among ACTB expression and tumor purity across LIHC, HNSC, and LUAD samples (**Figure 11C**).

Identifying ACTB expression regulators (miRNAs and TFs)

Via Enrichr analysis, a total of ten highly significant TFs (AATF, WWTR1, GFI1, NR3C1, MYC, GFI1, STAT3, ING1, MAX, and POU4F1) and ten miRNAs (hsa-miR-1908, hsa-miR-663, hsa-miR-744, hsa-miR-4745-3p, hsa-miR-1538, hsa-miR-3960, hsa-miR-4749-5p, hsa-miR-4706, hsa-miR-4743, and hsa-miR-3180-3p) were explored that could potentially change the ACTB expression (**Figure 12**). In sum, our results exhibited that a variety of different regulators (miRNAs and TFs) are involved in ACTB expression regulation.

Gene-drug interaction net-work

Via CTD analysis, we observed in this work that ACTB expression can potentially be influenced by a number of drugs. These predictions were made based on the experimental trials carried out by the previous studies. For example, valporic acid and metribolone could elevate the expression level of ACTB, while warfarin and aflotoxin B1 could reduce ACTB expression level (**Figure 13**).

Discussion

At present, cancer is one of the major lethal diseases worldwide [30]. In sum, there is an urgent

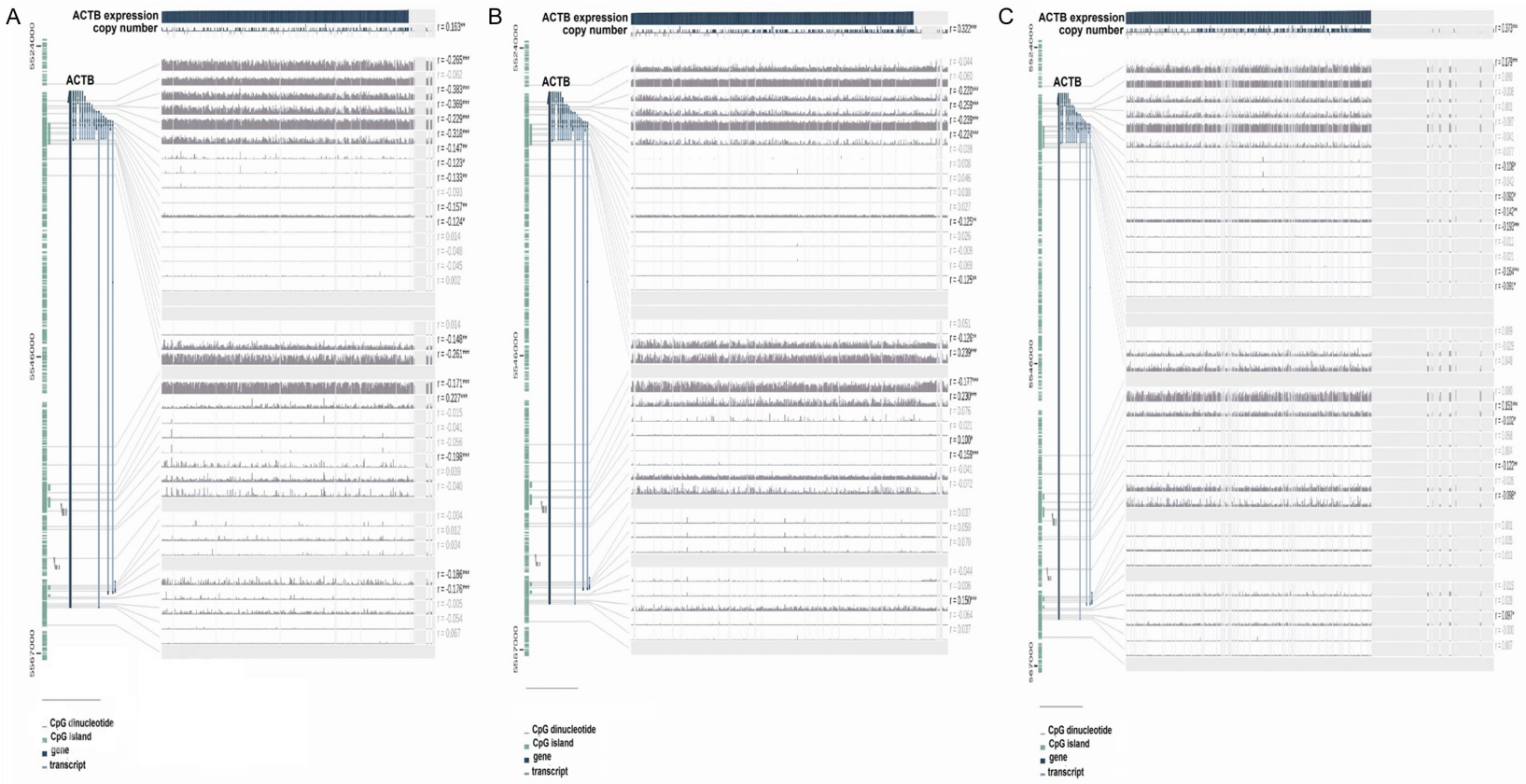


Figure 5. ACTB promoter methylation level (MEXPRESS). (A) Across LIHC, (B) Across HNSC, and (C) Across LUAD. $P < 0.05$.

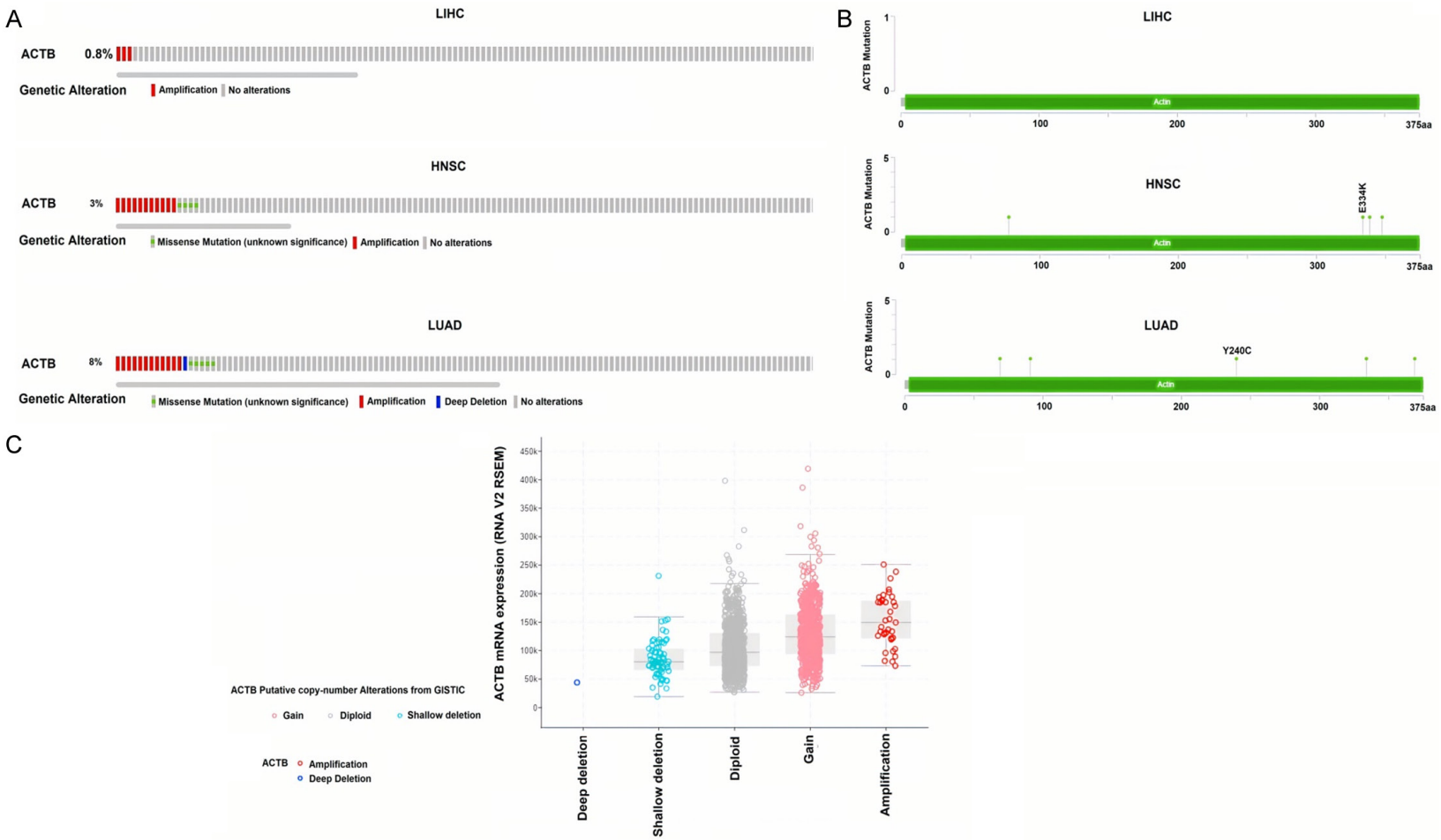


Figure 6. Genetic alteration landscape of ACTB (cBioPortal). (A) Percentage of LIHC, HNSC, and LUAD samples positive for ACTB genetic alterations, (B) Amino acids alteration landscape of ACTB observed mutations, and (C) Types of ACTB genetic alterations.

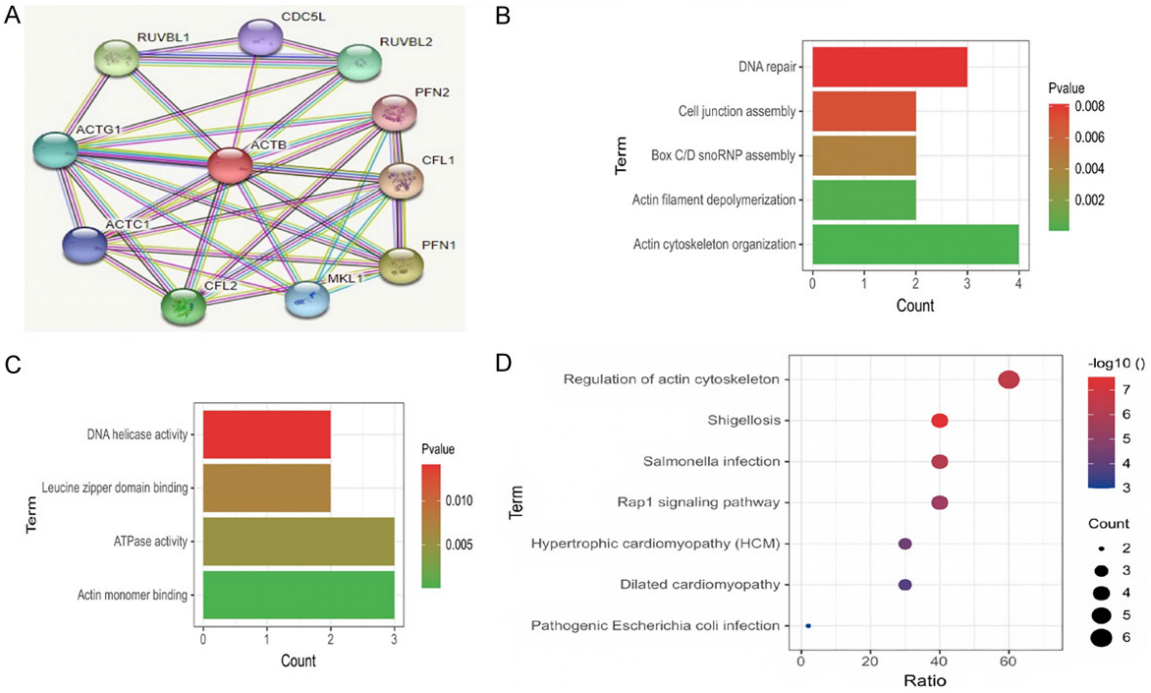


Figure 7. A PPI network and GO, and KEGG items of ACTB associated genes. (A) A PPI network, (B) BP GO items, (C) MF GO items, and (D) KEGG items. $P < 0.05$.

Table 1. Detail of Go and KEGG enrichment analysis of the ACTB enriched genes

Term ID	Name	Gene count	*P* value	Gene name
Detail of GO BP terms				
GO:0030036	Actin cytoskeleton organization	4	< 0.05	CFL1, MKL1, PFN1, PFN2
GO:0000492	Box C/D snoRNP assembly	2	< 0.05	RUVBL2, RUVBL1
GO:0030042	Actin filament depolymerization	2	< 0.05	CFL2, CFL1
GO:0034329	Cell junction assembly	2	< 0.05	ACTB, ACTG1
GO:0006281	DNA repair	3	< 0.05	RUVBL2, RUVBL1, CDC5L
Detail of GO MF terms				
GO:0003785	Actin monomer binding	3	< 0.05	MKL1, PFN1, PFN2
GO:0016887	ATPase activity	3	< 0.05	ACTC1, RUVBL2, RUVBL1
GO:0043522	Leucine zipper domain binding	2	< 0.05	MKL1, CDC5L
GO:0003678	DNA helicase activity	2	< 0.05	RUVBL2, RUVBL1
Detail of KEGG terms				
hsa04810	Regulation of actin cytoskeleton	6	< 0.05	CFL2, CFL1, PFN1, ACTB, PFN2, ACTG1
hsa05131	Shigellosis	4	< 0.05	PFN1, ACTB, PFN2, ACTG1
hsa05132	Salmonella infection	4	< 0.05	PFN1, ACTB, PFN2, ACTG1
hsa04015	Rap1 signaling pathway	4	< 0.05	PFN1, ACTB, PFN2, ACTG1
hsa05410	Hypertrophic cardiomyopathy (HCM)	3	< 0.05	ACTC1, ACTB, ACTG1
hsa05414	Dilated cardiomyopathy	3	< 0.05	ACTC1, ACTB, ACTG1
hsa05130	Pathogenic Escherichia coli infection	2	< 0.05	ACTB, ACTG1

need to discover new biomarkers for dealing with cancer diagnosis and treatment.

β-actin protein is encoded by the ACTB gene, which mainly expresses in nonmuscle cells and assists in the cytoskeleton's construction and movement of the cells. ACTB performs a variety of functions as a regulatoryprotein, including cell growth maintenance, cell migration, cell differentiation, and signal transduction [31].

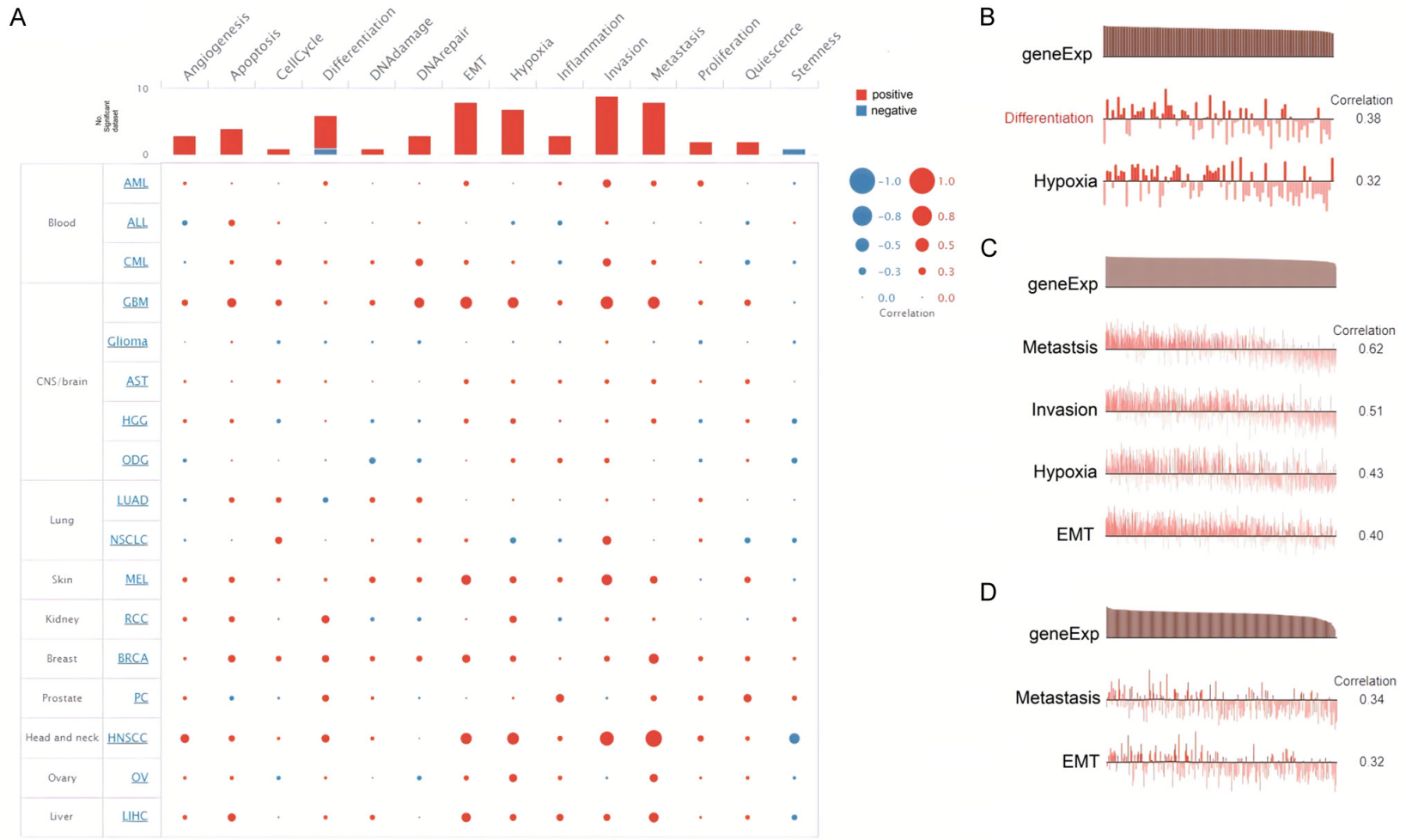


Figure 8. ACTB functional analysis at single cell level (CancerSEA). (A) ACTB enriched genes associated 14 different functional states, (B) ACTB enriched genes associated significant functional states in LIHC, (C) ACTB enriched genes associated significant functional states in HNSC, and (D) ACTB enriched genes associated significant functional states in LUAD. Red and blue color dotes present a positive correlation and a negative correlation, respectively, while the depth of the color indicates the average of correlation. $P < 0.05$.

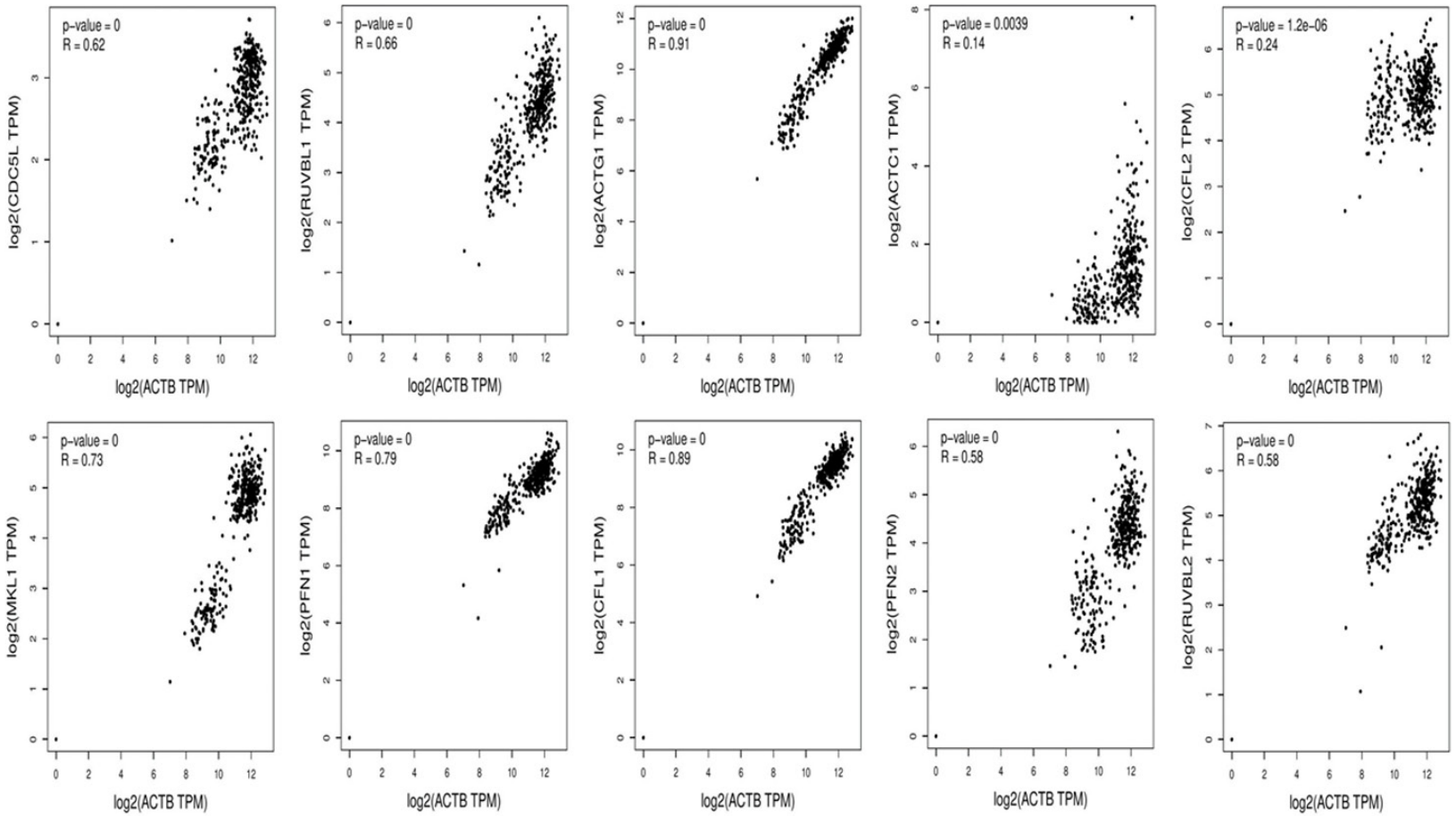


Figure 9. Correlations among ACTB expression and its other associated genes across LIHC, HNSC, and LUAD (GEPIA). $P < 0.05$.

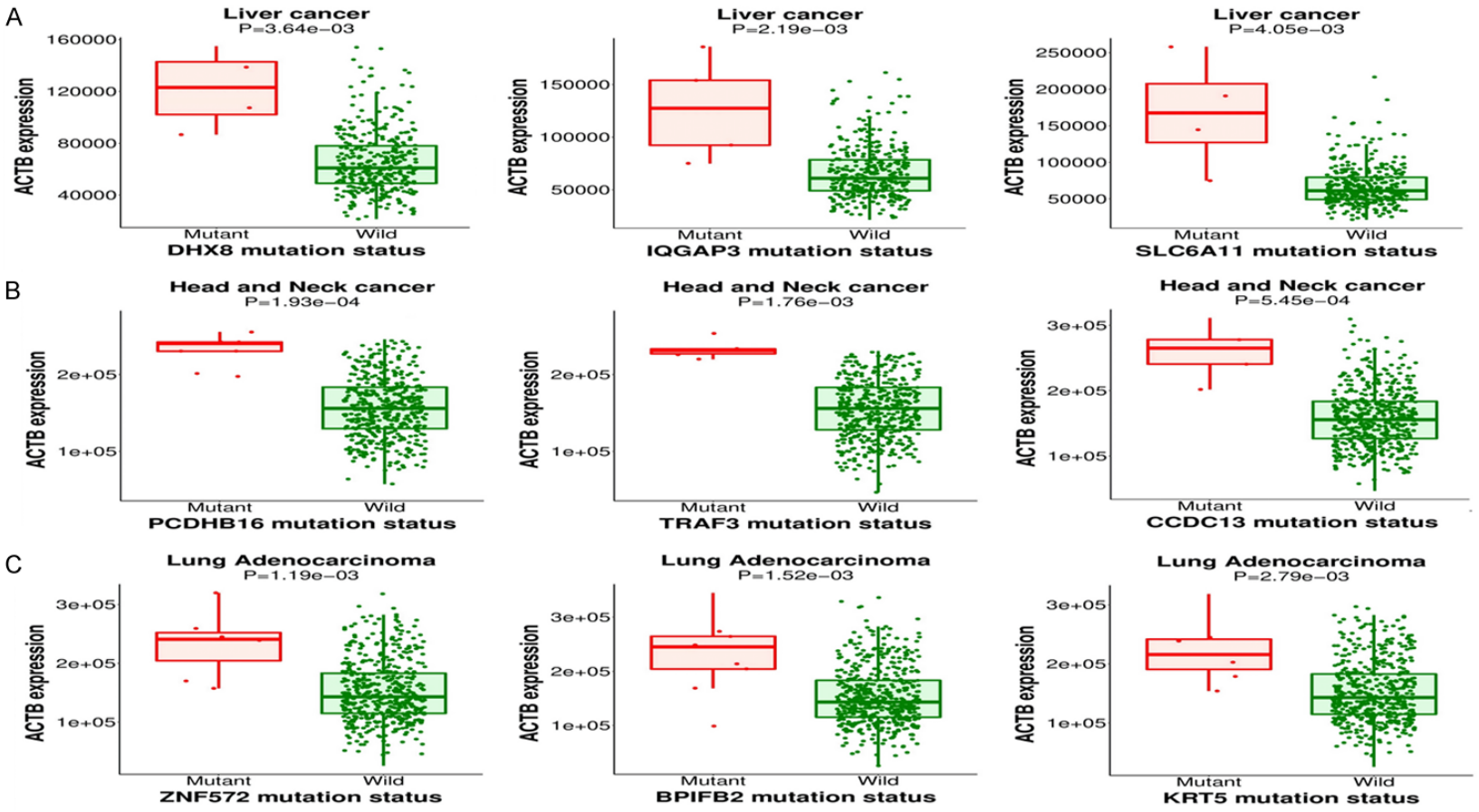


Figure 10. ACTB positively correlated mutant genes (MuTarget). (A) ACTB correlated top 3 gene across LIHC, (B) ACTB correlated top 3 gene across HNSC, and (C) ACTB correlated top 3 gene across in LUAD. $P < 0.05$.

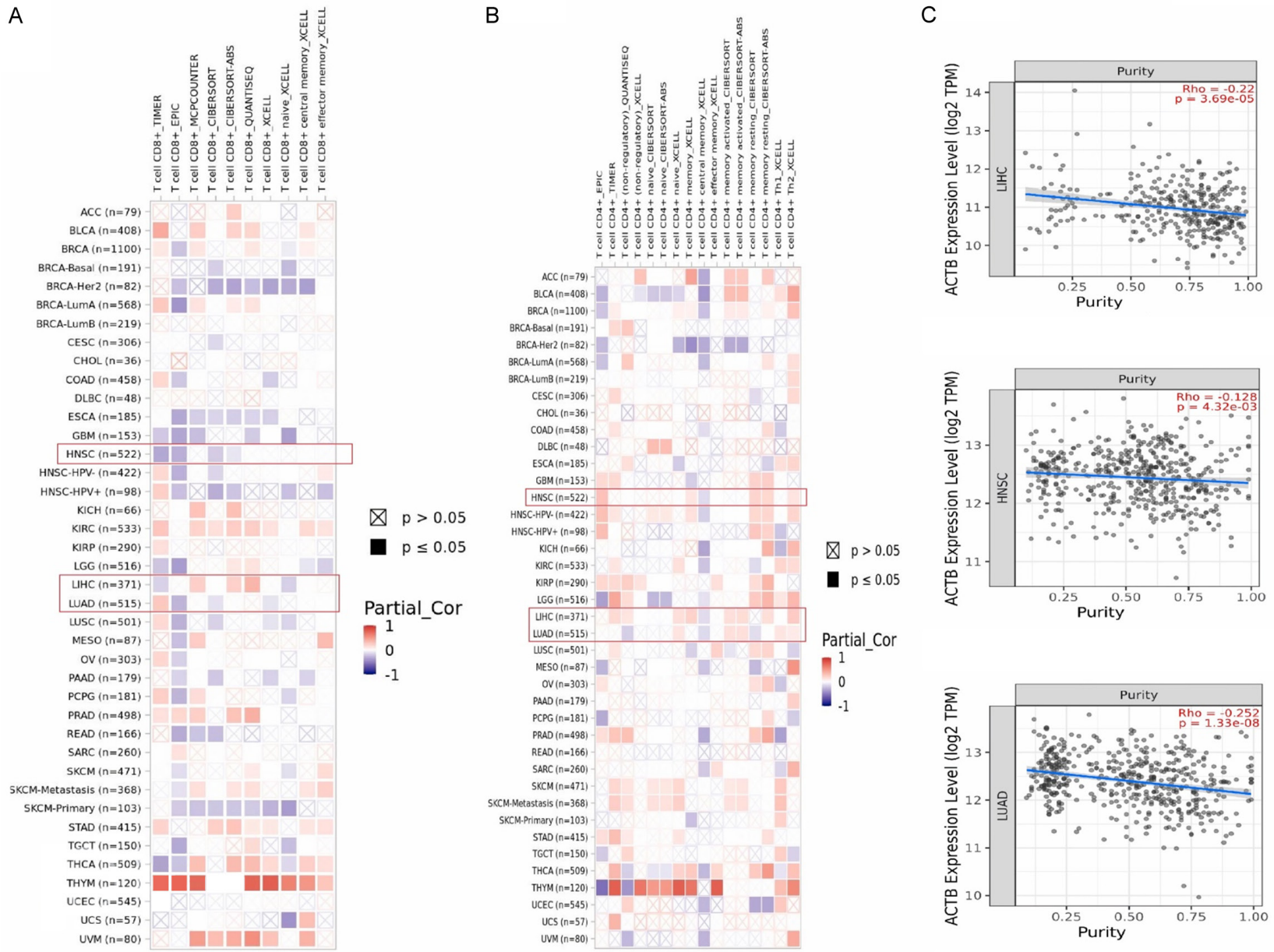
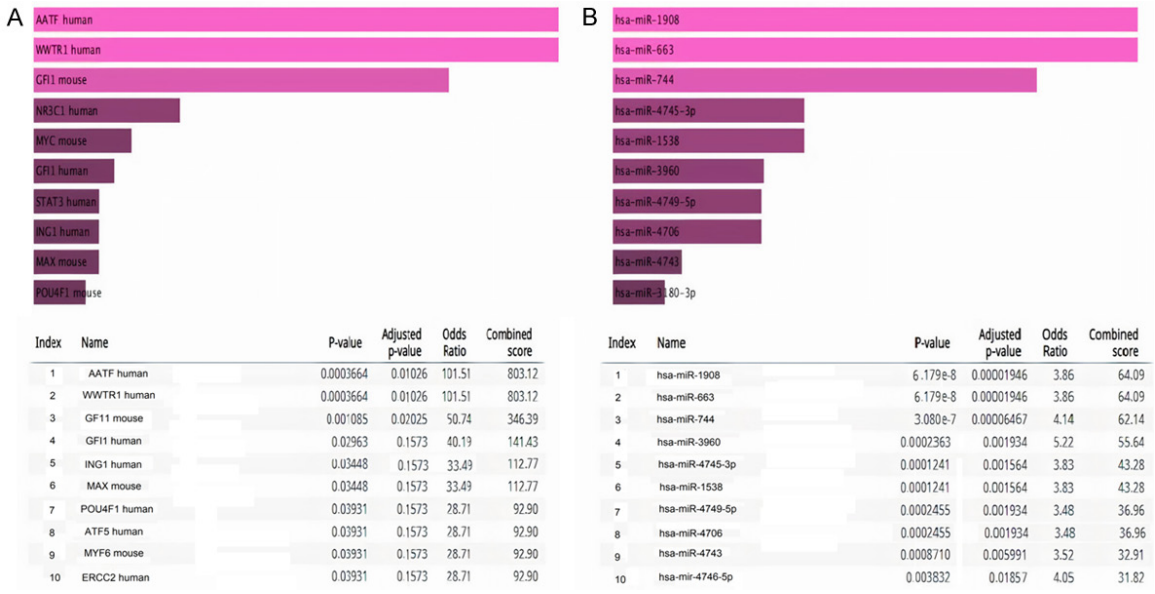


Figure 11. ACTB correlation with tumor purity, immune cells (CD4+ T and CD8+ T), and ACTB expression (TIMER). (A) With CD4+ T immune cells, (B) With CD8+ T, and (C) With tumor purity. $P < 0.05$.



Index	Name	P-value	Adjusted p-value	Odds Ratio	Combined score
1	AATF human	0.0003664	0.01026	101.51	803.12
2	WWTR1 human	0.0003664	0.01026	101.51	803.12
3	GF11 mouse	0.001085	0.02025	50.74	346.39
4	GFI1 human	0.02963	0.1573	40.19	141.43
5	ING1 human	0.03448	0.1573	33.49	112.77
6	MAX mouse	0.03448	0.1573	33.49	112.77
7	POU4F1 human	0.03931	0.1573	28.71	92.90
8	ATF5 human	0.03931	0.1573	28.71	92.90
9	MYF6 mouse	0.03931	0.1573	28.71	92.90
10	ERCC2 human	0.03931	0.1573	28.71	92.90

Index	Name	P-value	Adjusted p-value	Odds Ratio	Combined score
1	hsa-miR-1908	6.179e-8	0.00001946	3.86	64.09
2	hsa-miR-663	6.179e-8	0.00001946	3.86	64.09
3	hsa-miR-744	3.080e-7	0.00006467	4.14	62.14
4	hsa-miR-3960	0.0002363	0.001934	5.22	55.64
5	hsa-miR-4745-3p	0.0001241	0.001564	3.83	43.28
6	hsa-miR-1538	0.0001241	0.001564	3.83	43.28
7	hsa-miR-4749-5p	0.0002455	0.001934	3.48	36.96
8	hsa-miR-4706	0.0002455	0.001934	3.48	36.96
9	hsa-miR-4743	0.0008710	0.005991	3.52	32.91
10	hsa-mir-4746-5p	0.003832	0.01857	4.05	31.82

Figure 12. Prediction of ACTB regulatory TFS and miRNAs. (A) Predicted miRNAs, and (B) Predicted TFs. $P < 0.05$.

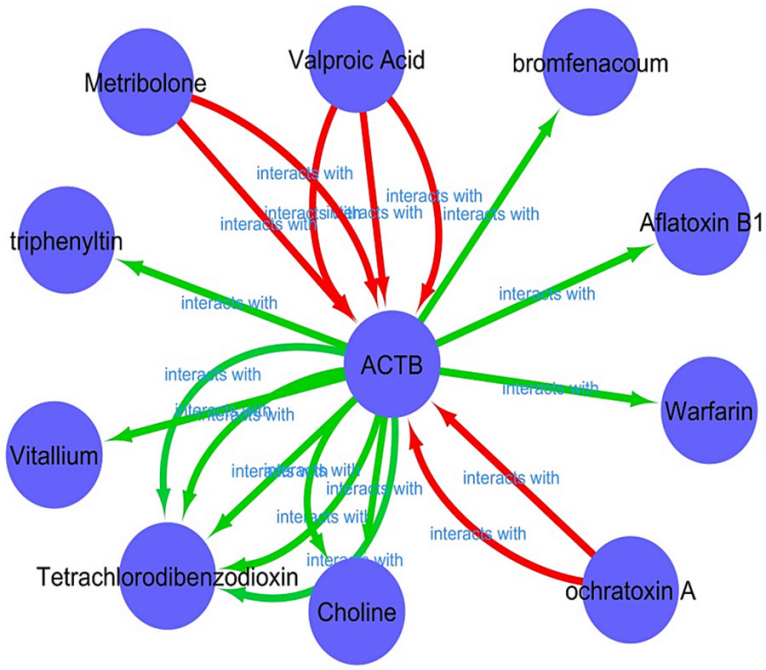


Figure 13. A Gene-drug interaction network of ACTB (CTD). Red arrows: drugs that can increase ACTB transcription expression; green arrows: different drugs that can decrease transcription expression. The numbers of arrows represent the supported numbers of study to a specific interaction.

Different studies [32-34] have already documented the role of ACTB in the development of distinct tumor types. For example, Lim et al. [32], during their clinical experiences have reported that ACTB mutations may lead to the development of pilocytic astrocytoma. The ACTB glioma-associated oncogene homolog 1 (GLI1) fusion has also been identified as a genetic abnormality that could lead to the distinctive type of actin-positive such as perivascular myoid tumors, also known as "pericytoma with t(7;12) translocation" [33]. Furthermore, Castro et al. [34] stated that ACTB and GLI1 fusion can also develop a gastric tumor that arises from the pyloric wall of the stomach. Another study by Zhang et al. revealed the lower ACTB expression across LUAD samples and also suggested it as a worst prognostic factor [35].

In this study, we revealed that ACTB was up-regulated in 24 subtypes of human cancers and its overexpression was significantly ($P < 0.05$) correlated with the decreased OS duration, RFS duration, and advanced metastasis in LIHC, HNSC, and LUAD patients. The ACTB oncogenic role was examined earlierusing wet-lab experiments in previously published studies, and it

was discovered that higher ACTB expression was linked to a variety of altered mechanisms in cancer cells. For instance, in tumour cells, overexpression of ACTB results in cell invasion [36]. Beside this, higher ACTB protein level is a major contributing factor in renal cell carcinoma development [37]. Moreover, the higher ACTB mRNA expression was also predictive to be the causative agent of hepatocellular carcinoma (HCC) [38-40].

Earlier studies highlighted that genomic alterations such as genomic mutations found frequently in cancer patients [41]. Moreover, it is also well known that promoter methylation level abnormalities contribute significantly to the gene expression regulation, DNA repair, and replication processes [41, 42]. In this work, we analyzed ACTB promoter methylation and genetic changes across LIHC, HNSC, and LUAD samples. Results of this analysissuggested that hypomethylation contributes majorly while genetic mutations participate at a minor level to affect ACTB expression.

Understanding tumour purity and immune cell infiltration is critical for developing effective anticancer immunity [43]. The interesting positive andnegative correlations observed in our studybetween ACTB expression and tumor purity, CD8+ T, and CD4+ T immune cells may bring new ideas for the treatment of LIHC, HNSC, and LUAD.

Recent studies have explored that TFS and miRNAs directly regulate gene expression [44, 45]. In this study, we identified the different TFS and miRNAs that can regulate ACTB expression. Based on the prediction analysis, following ten most significant TFS: AATF, WWTR1, GFI1 [mouse], NR3C1, MYC, GFI1, STAT3, ING1, MAX, and POU4F1 and ten miRNAs: hsa-miR-1908, hsa-miR-663, hsa-miR-744, hsa-miR-4745-3p, hsa-miR-1538, hsa-miR-3960, hsa-miR-4749-5p, hsa-miR-4706, hsa-miR-4743, and hsa-miR-3180-3p can potentially regulate ACTB expression. In view of these results, we think that regulation of ACTB via these TFS and miRNAs might be another new mechanism of LIHC, HNSC, and LUAD development. However, additional work should be done to verify this hypothesis.

So far, LIHC, HNSC, and LUAD patients carrying different mutant genes do not have any appropriate therapy. In view of this, using muTarget, we explored different mutant genes that are associated with altered ACTB expression. As a result, we have identified 3 top mutant genes in each LIHC, HNSC, and LUAD, respectively, for ACTB, including DHX8, IQGAP3, and SLC6A11 in LIHC, PCDHB16, TRAF3, and CCDC13 in HNSC, and ZNF572, BPIFB2, and KRT5 in LUAD.

By exploring and linking these mutant genes with ACTB expression, it is convenient to identify potential mutant gene-based therapies for LIHC, HNSC, and LUAD patients.

Moreover, according to the previous studies concerning CNVs and genetic mutations in the ACTB gene, the amplification of this gene was explored as the most prominent type of CNVs across uterine carcinosarcoma, esophageal adenocarcinoma and adrenocortical carcinoma [46]. While the occurrence of genetic mutation in the ACTB gene across different cancers was a less frequent phenomenon. Recently, a pan-cancer study detected a total of 129 mutations in the ACTB gene across different cancer subtypes, such as esophageal adenocarcinoma and adrenocortical carcinoma, and most of these mutations were missense [47].

A PPI revealed a set of ten ACTB-associated genes involved in “Actin cytoskeleton organization, Box C/D snoRNP assembly, Actin filament depolymerization, Cell junction assembly, and DNA repair biological process (BP)” GO terms, “Actin monomer binding, ATPase activity, Leucine zipper domain binding, DNA helicase activity molecular function (MF)” terms, and “Regulation of actin cytoskeleton, Shigellosis”, etc. KEGG terms.

In order to confirm ACTB role in cancer development, we analyzed the 14 functional states of this gene in LIHC, HNSC, and LUAD using CancerSEA. Results of the analysis found that ACTB was significantly correlated with differentiation and hypoxia in LIHC, metastasis, invasion, hypoxia, EMT in HNSC, and metastasis and EMT in LUAD. Best to our knowledge, this study is the first to explore the role of ACTB in differentiation and hypoxia in LIHC, metastasis, invasion, hypoxia, EMT in HNSC, and metastasis and EMT in LUAD.

Although the results of this study have numerous advantages, there are still some draw-

backs. Bioinformatics analysis provided the primary foundation for our conclusions. Utilizing internal in-house clinical samples to validate ACTB expression could significantly reinforce our conclusions.

Conclusion

We confirmed that ACTB was overexpressed in LIHC, HNSC, and LUAD samples and can be a possible diagnostic and prognostic biomarker of survival in these cancer patients. However, additional experiments should be conducted for further verification.

Acknowledgements

The authors extend their appreciation to the Researchers Supporting Project number (RSPD2023R725) King Saud University, Riyadh, Saud Arabia.

Disclosure of conflict of interest

None.

Address correspondence to: Mostafa A Abdel-Maksoud, Botany and Microbiology Department, College of Science, King Saud University, Riyadh 11451, Saudi Arabia. E-mail: Mabdelmaksoud@ksu.edu.sa

References

[1] Wang H, Naghavi M, Allen C, Barber RM, Bhutta ZA, Carter A, Casey DC, Charlson FJ, Chen AZ and Coates MM. Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980-2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet 2016; 388: 1459-1544.

[2] Siegel RL, Miller KD and Jemal A. Cancer statistics, 2019. CA Cancer J Clin 2019; 69: 7-34.

[3] Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA and Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018; 68: 394-424.

[4] Weir HK, Thompson TD, Soman A, Møller B and Leadbetter S. The past, present, and future of cancer incidence in the United States: 1975 through 2020. Cancer 2015; 121: 1827-1837.

[5] You W and Henneberg M. Cancer incidence increasing globally: the role of relaxed natural selection. Evol Appl 2018; 11: 140-152.

[6] Hanahan D and Weinberg RA. The hallmarks of cancer. Cell 2000; 100: 57-70.

[7] Vogelstein B and Kinzler KW. Cancer genes and the pathways they control. Nat Med 2004; 10: 789-799.

[8] Siegel RL, Miller KD and Jemal A. Cancer statistics, 2016. CA Cancer J Clin 2016; 66: 7-30.

[9] Zafar SY, Peppercorn JM, Schrag D, Taylor DH, Goetzinger AM, Zhong X and Abernethy AP. The financial toxicity of cancer treatment: a pilot study assessing out-of-pocket expenses and the insured cancer patient's experience. Oncologist 2013; 18: 381.

[10] Hwang TJ, Kesselheim AS and Gyawali B. Affordability and price increases of new cancer drugs in clinical guidelines, 2007-2016. JNCI Cancer Spectr 2018; 2: pky016.

[11] Guo C, Liu S, Wang J, Sun MZ and Greenaway FT. ACTB in cancer. Clin Chem 2013; 417: 39-44.

[12] Chandrashekar DS, Bashel B, Balasubramanya SAH, Creighton CJ, Ponce-Rodriguez I, Chakravarthi BVSK and Varambally S. UALCAN: a portal for facilitating tumor subgroup gene expression and survival analyses. Neoplasia 2017; 19: 649-658.

[13] Maciejczyk A, Szelachowska J, Czapiga B, Matkowski R, Hałoń A, Györffy B and Surowiak P. Elevated BUBR1 expression is associated with poor survival in early breast cancer patients: 15-year follow-up analysis. J Histochem Cytochem 2013; 61: 330-339.

[14] Bartha Á and Győrffy B. TNMplot.com: a web tool for the comparison of gene expression in normal, tumor and metastatic tissues. Int J Mol Sci 2021; 22: 2622.

[15] Park SJ, Yoon BH, Kim SK and Kim SY. GENT2: an updated gene expression database for normal and tumor tissues. BMC Med Genomics 2019; 12: 101.

[16] Li T, Fan J, Wang B, Traugh N, Chen Q, Liu JS, Li B and Liu XS. TIMER: a web server for comprehensive analysis of tumor-infiltrating immune cells. Cancer Res 2017; 77: e108-e110.

[17] Tang Z, Li C, Kang B, Gao G, Li C and Zhang Z. GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. Nucleic Acids Res 2017; 45: W98-W102.

[18] Thul PJ and Lindskog C. The human protein atlas: a spatial map of the human proteome. Protein Sci 2018; 27: 233-244.

[19] Clough E and Barrett T. The gene expression omnibus database. Methods Mol Biol 2016; 1418: 93-110.

[20] Koch A, De Meyer T, Jeschke J and Van Criekinge W. MEXPRESS: visualizing expression, DNA methylation and clinical TCGA data. BMC Genomics 2015; 16: 636.

[21] Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, Jacobsen A, Byrne CJ, Heuer ML, Larsson E, Antipin Y, Reva B, Goldberg AP, Sander C and Schultz N. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov 2012; 2: 401-404.
[22] von Mering C, Huynen M, Jaeggi D, Schmidt S, Bork P and Snel B. STRING: a database of predicted functional associations between proteins. Nucleic Acids Res 2003; 31: 258-261.
[23] Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B and Ideker T. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res 2003; 13: 2498-2504.
[24] Huang DW, Sherman BT, Tan Q, Collins JR, Alvord WG, Roayaei J, Stephens R, Baseler MW, Lane HC and Lempicki RA. The DAVID gene functional classification tool: a novel biological module-centric algorithm to functionally analyze large gene lists. Genome Biol 2007; 8: R183.
[25] Yuan H, Yan M, Zhang G, Liu W, Deng C, Liao G, Xu L, Luo T, Yan H, Long Z, Shi A, Zhao T, Xiao Y and Li X. CancerSEA: a cancer single-cell stateatlas. Nucleic Acids Res 2019; 47: D900-D908.
[26] Nagy Á and Győrffy B. muTarget: a platform linking gene expression changes and mutation status in solid tumors. Int J Cancer 2021; 148: 502-511.
[27] Li T, Fu J, Zeng Z, Cohen D, Li J, Chen Q, Li B and Liu XS. TIMER2.0 for analysis of tumor-infiltrating immune cells. Nucleic Acids Res 2020; 48: W509-W514.
[28] Kuleshov MV, Jones MR, Rouillard AD, Fernandez NF, Duan Q, Wang Z, Koplev S, Jenkins SL, Jagodnik KM, Lachmann A, McDermott MG, Monteiro CD, Gundersen GW and Ma'ayan A. Enrichr: a comprehensive gene set enrichment analysis web server 2016 update. Nucleic Acids Res 2016; 44: W90-W97.
[29] Mattingly CJ, Colby GT, Forrest JN and Boyer JL. The comparative toxicogenomics database (CTD). Environ Health Perspect 2003; 111: 793-795.
[30] Nagai H and Kim YH. Cancer prevention from the perspective of global cancer burden patterns. J Thorac Dis 2017; 9: 448-451.
[31] Pavlyk I, Leu NA, Vedula P, Kurosaka S and Kashina A. Rapid and dynamic arginylation of the leading edge β-actin is required for cell migration. Traffic 2018; 19: 263-272.
[32] Lim YH, Burke AB, Roberts MS, Collins MT and Choate KA. Multilineage ACTB mutation in a patient with fibro-osseous maxillary lesion and pilocytic astrocytoma. Am J Med Genet A 2018; 176: 2037-2040.
[33] Antonescu CR, Agaram NP, Sung YS, Zhang L, Swanson D and Dickson BC. A distinct malignant epithelioid neoplasm with GLI1 gene rearrangements, frequent S100 protein expression and metastatic potential: expanding the spectrum of pathologic entities with ACTB1/MALAT1/PTCH1-GLI1 fusions. Am J Pathol 2018; 42: 553.
[34] Castro E, Cortes-Santiago N, Ferguson LM, Rao PH, Venkatramani R and López-Terrada D. Translocation t (7;12) as the sole chromo-somal abnormality resulting in ACTB-GLI1 fusion in pediatric gastric pericytoma. Hum Pathol 2016; 53: 137-141.
[35] Zhang N and Zhang SW. Identification of differentially expressed genes between primary lung cancer and lymph node metastasis via bioinformatic analysis. Oncol Lett 2019; 18: 3754-3768.
[36] Le PU, Nguyen TN, Drolet-Savoie P, Leclerc N and Nabi IR. Increased β-actin expression in an invasive moloney sarcoma virus-transformed MDCK cell variant concentrates to the tips of multiple pseudopodia. Cancer Res 1998; 58: 1631-1635.
[37] Ferguson RE, Carroll HP, Harris A, Maher ER, Selby PJ and Banks RE. Housekeeping proteins: a preliminary study illustrating some limitations as useful references in protein expression studies. Proteomics 2005; 5: 566-571.
[38] Gao Q, Wang XY, Fan J, Qiu SJ, Zhou J, Shi YH, Xiao YS, Xu Y, Huang XW and Sun J. Selection of reference genes for real-time PCR in human hepatocellular carcinoma tissues. J Cancer Res Clin Oncol 2008; 134: 979-986.
[39] Fu LY, Jia HL, Dong QZ, Wu JC, Zhao Y, Zhou HJ, Ren N, Ye QH and Qin LX. Suitable reference genes for real-time PCR in human HBV-related hepatocellular carcinoma with different clinical prognoses. BMC Cancer 2009; 9: 49.
[40] Li Y, Ma H, Shi C, Feng F and Yang L. Mutant ACTB mRNA 3'-UTR promotes hepatocellular carcinoma development by regulating miR-1 and miR-29a. Cell Signal 2020; 67: 109479.
[41] Michalak EM, Burr ML, Bannister AJ and Dawson MA. The roles of DNA, RNA and histone methylation in ageing and cancer. Nat Rev Mol Cell Biol 2019; 20: 573-589.
[42] Palangat M, Anastasakis DG, Fei DL, Lindblad KE, Bradley R, Hourigan CS, Hafner M and Larson DR. The splicing factor U2AF1 contributes to cancer progression through a noncanonical role in translation regulation. Genes Dev 2019; 33: 482-497.
[43] Riazi Rad F, Ajdary S, Omranipour R, Alimohammadian MH and Hassan ZM. Comparative analysis of CD4+ and CD8+ T cells in tumor tissues, lymph nodes and the peripheral blood from patients with breast cancer. Iran BiomedJ 2015; 19: 35-44.

[44] Waheed S and Zeng L. The critical role of miRNAs in regulation of flowering time and flowerdevelopment. Genes (Basel) 2020; 11: 319.
[45] Martinez NJ and Walhout AJM. The interplay between transcription factors and microRNAs in genome-scale regulatory networks. Bioessays 2009; 31: 435-445.
[46] Khan SA, Tyagi M, Sharma AK, Barreto SG, Sirohi B, Ramadwar M, Shrikhande SV and Gupta S. Cell-type specificity of β-actin expression and its clinicopathological correlation in gastric adenocarcinoma. World J Gastroenterol 2014; 20: 12202-11.
[47] Gu Y, Tang S, Wang Z, Cai L, Lian H, Shen Y and Zhou Y. A pan-cancer analysis of the prognostic and immunological role of β-actin (ACTB) in human cancers. Bioengineered 2021; 12: 6166-6185.